

FEEDING TESTS WITH *INDIGOFERA ENDECAPHYLLA* JACQ.  
(CREEPING INDIGO) AND SOME OBSERVATIONS ON ITS  
POISONOUS EFFECTS ON DOMESTIC ANIMALS

SAM NORDFELDT, L. A. HENKE, K. MORITA,  
H. MATSUMOTO, M. TAKAHASHI, AND O. R. YOUNGE

*Hawaii Agricultural Experiment Station  
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E. H. WILLERS AND R. F. CROSS

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UNIVERSITY OF HAWAII  
COLLEGE OF AGRICULTURE  
AGRICULTURAL EXPERIMENT STATION  
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## INTRODUCTION

Leguminous plants have long been considered important as livestock feeds in Hawaii. They decrease the need for costly oil cakes and protein-rich concentrates in milk and meat production.

*Indigofera endecaphylla* Jacq. (creeping indigo or trailing indigo) (11) seemed promising for a time as a high-rainfall-zone legume. Early experiments proved that it would grow well with a wide variety of associated grasses, and grazing tests showed that it was palatable and quite persistent under pasture conditions.

Very little was known, on the other hand, of its feeding value for livestock. In 10 years of short-interval pasture trials with relatively small proportions of the legume, no adverse effects were noted on young cattle. However, when the concentration of the legume exceeded about 50 percent of the forage, toxicity symptoms began to appear. In early 1949, two heifers on lush creeping indigo pastures showed the characteristic reaction of walking in circles with heads low. These manifestations led to the investigations reported here.

### DAIRY COWS AND BRED HEIFERS

During the feed shortage induced by the shipping strike in Hawaii in 1949, creeping indigo was fed green and unchopped to nine bred heifers and seven dry cows on dry sparse pasture. This feeding was begun July 9 and discontinued August 11. As reported elsewhere (11), two normal calves were born after 20 days on creeping indigo and sparse pasture. From the 25th day on, six calves either were born dead or lived only a few hours. Four of the calves were born 30 to 50 days premature. The fifth was born at normal time, 3 days after indigo feeding ceased. Five days after delivery, the mother of this calf died. The sixth calf was born 7 days after indigo feeding was stopped, apparently more than 3 months premature.

In March, 1950, an experiment was initiated to determine the toxicity of creeping indigo in mixed pasture under grazing. Two bred heifers were to be grazed continuously in quarter-acre paddocks in which three species of grasses—Guinea grass (*Panicum maximum*), Napier grass (*Pennisetum purpureum*), and Dallis grass (*Paspalum dilatatum*)—had been interplanted with creeping indigo.

The heifers were in Guinea grass-creeping indigo pasture for 20 days, after which they were in Napier grass-creeping indigo pasture for 24 days. They were then transferred to Dallis grass-creeping indigo pasture. The proportions of creeping indigo on fresh weight basis in the various mixtures were as follows: (1) Guinea grass-creeping indigo paddock, 15.90 percent; (2) Napier grass-creeping indigo paddock, 26.86 percent; and (3) Dallis grass-creeping indigo, 52.38 percent.

Both heifers appeared to be in good condition after 44 days of grazing in paddocks (1) and (2). One heifer aborted on the 11th day and the second on the 19th day after being placed in paddock (3).

### NON-BRED HEIFERS

A 15-day trial with five non-bred heifers was carried out in July 1949. Starting at a 30 percent level, increasing proportions of chopped, fresh creep-

TABLE 1. Average daily feed consumption and gain in weight of heifers fed chopped creeping indigo.

	AVERAGE DAILY FEED CONSUMPTION PER HEIFER						BREEDING RECORD AND WEIGHT INCREASE			
	Creeping indigo	Concentrate mixture		Copra	Molasses	Bred	Heifer 308		Heifer 309	
	lbs.	no.	lbs.	lbs.	lbs.		Daily weight increase	Bred	Daily weight increase	Bred
1949										
July.....	25.5	13	3.0		3					
August.....	27.4	12	5.4		3			8/24	1.15	
September.....	19.6	7	6		3	9/24	1.0		2.19	
October.....	17.2	7	6		3		-0.85*		1.31	
November.....	16.6	7	6		3		-0.97		-0.36	
December.....	17.3	7	6		3		2.00	11/16	0.79	
1950							2.75			
January.....	24.9	7	6	0.40	3		0.73		1.27	
February.....	23.4	7	6	0.50	3		0.93		0.71	
March.....	21.7	7	7	0.10	3		0.97		1.03	3/15
April.....	21.6	7	7		3	4/25	2.34		2.57	
May.....	20.3	12	7		3		0.56		0.44	
June.....	12.9	12	7		3		0.34		-0.06**	6/22
July.....	8.8	12	7		3		2.17		0.24	
Initial weight of heifers 7/19/49.....							620		718	
Final weight of heifers 7/18/50.....							983		1063	
Average 12 months.....	21.4		6.6	0.08	3		0.99		0.85	
Control heifers										
average 6 months	Napier									
7/19-12/13.....	grass									
	34.6		6.0		3		#304		#306	
							0.95		1.09	
										#307
										1.31

\* Negative signs mean loss of weight.

\*\* Heifer 309 aborted June 14.

ing indigo mixed with chopped Napier grass were fed until, after 10 days of feeding, the ration consisted entirely of creeping indigo. All animals gradually developed anorexia (loss of appetite) and lost weight.

On July 18, 1949, three non-bred heifers (Nos. 308, 309, and 311) were started on a ration of chopped creeping indigo. The heifers were born on the following dates: No. 308 on January 13, 1948; No. 309 on March 16, 1948; and No. 311 on May 17, 1948. The age of the heifers at the start of the experiment, therefore, was 13 to 18 months. This feeding experiment was carried on for 12 months but had to be discontinued due to pressure of other work. The heifers were weighed at 2-week intervals throughout the year. The feed consumption was also controlled. Consumption of the legume alone was low, and extra feed had to be supplied to prevent complications from lowered intake. The average daily consumption and the growth of the heifers are recorded in table 1, and the calculated chemical compositions of feeds used are given in table 2.

In table 1 the growth of three heifers fed a normal diet of Napier grass is also reported. The data for these control heifers include only the first 6-month period of the experiment, because during the latter half of the year their growth became abnormal as they entered periods of pregnancy and parturition.

The table shows that the growth of heifer No. 311 was definitely influenced by the feeding of creeping indigo. For 12 months her increase in weight was only 64 pounds, i.e., 0.18 pound daily, or almost a standstill in growth. This poor growth was probably due to decreased appetite. Heifer No. 308 gained about 1 pound daily, and No. 309 averaged 0.85 pound daily. However, the growth was uneven, and there were periods with no growth. For example, No. 308 lost weight during September and October 1949, as did heifer No. 309 during November 1949. During other months the growth varied, but the yearly average was about the same as for heifers fed on Napier grass. Temperature, pulse, and respiration were normal.

TABLE 2. Chemical composition of feeds used in creeping indigo feeding tests.

FEED	DRY MATTER	T.D.N.	DIGEST- IBLE PRO- TEIN	TOTAL				
				Protein	Ether extract	Fiber	N-free matter	Ash
Heifer mixture 7.....	85.81	75.30	14.74	18.41	11.69	7.99	40.08	7.64
Heifer mixture 12.....	85.67	73.46	16.04	19.98	7.91	8.12	41.35	7.32
Heifer mixture 13.....	86.08	71.33	12.87	16.29	2.52	7.65	52.77	6.05
Copra.....	90.10	123.51	5.41	7.21	61.32	4.69	14.80	2.13
Molasses.....	79.70	59.67	0.73	2.27	...	...	66.26	11.16
Napier grass.....	24.43	14.61	0.77	1.28	0.47	9.02	10.68	2.98
Fresh creeping indigo leaf and stem.....	20.00	13.96	1.95	2.60	0.45	6.69	9.20	1.06
Semi-dry creeping indigo..	57.10	33.91	5.57	7.43	1.29	19.09	26.27	3.01
Commercial rabbit ration*	85.30	...	...	15.00	2.50	21.00	35.30	11.50

\* Analysis as given by manufacturer, dry matter determination made by Hawaii Station.

All three heifers were bred during the experiment, but none became pregnant from the first service. Heifer No. 309 became pregnant after the

second service but aborted 72 days before normal time. Thus, a total of nine dead or stillborn calves were produced after experimental feeding of creeping indigo.

Heifers No. 308 and No. 311 were rebred before the end of the experiment. Heifer No. 311 conceived and dropped a normal female calf 9 months later. Heifers No. 308 and No. 309 were rebred again and became pregnant 1½ months after the experiment. All heifers were negative to the agglutination test for brucellosis.

### SHEEP

On January 3, 1950, two male Merino sheep, weighing 72 and 65 pounds, were started on rations containing fresh creeping indigo. In addition to the legume, 0.5 pound concentrate was fed each animal daily. The concentrate consisted of 15 percent cane molasses, 45 percent rolled barley, 38 percent soybean oil meal, 1 percent salt, and 1 percent bone meal. In the beginning appetite was good, but after 2 weeks anorexia developed. At first, 4 pounds of creeping indigo was consumed per animal, but later 1 pound or less was eaten. Both sheep developed a complete or partial bilateral corneal opacity. Loss of weight occurred. One sheep died after 28 days. The other sheep exhibited general weakness and unsteady gait and was euthanatized after 38 days.

On May 22 two female sheep, a Suffolk weighing 171 pounds and a Corriedale weighing 95 pounds, were started on an experiment with fresh creeping indigo. As before, 0.5 pound of the concentrate mixture was fed in addition to the legume. The consumption of indigo was low and varied from 2 to 4 pounds per animal daily during the first 10 days of the experiment. After 2 weeks the consumption was 1 to 2 pounds daily, and animals lost weight. During the last few days of life, the animals refused to eat the concentrate mixture also.

The Corriedale ewe became sluggish and tired after 22 days on experiment. There was a purulent nasal and serous ocular discharge. The head was kept low and pressed against the fence. She developed no noticeable corneal opacity. After 26 days, she died.

The Suffolk sheep was pregnant and aborted after feeding 1 day on the legume. She was taken out of the experiment to avoid complications due to the abortion. After 6 days she appeared normal and was again started on the creeping indigo feed. As the feeding trial progressed, she became very weak, developed a severe diarrhea and a beginning corneal opacity, and was euthanatized after 38 days on experiment.

### PIGS

Fresh creeping indigo forage was also fed to growing pigs, but they refused to eat it.

### RABBITS

Experiments were carried out with young rabbits, mature animals, and pregnant does. The legume was fed in the fresh, green state or as a dehydrated leaf meal.

*Green, Fresh Creeping Indigo*

The legume was fed ad libitum to two mature bucks and two does of the New Zealand breed. All animals died within 30 days. One buck died after 6 days. Appetite was good at first but poor later. Consumption was 0.4 to 0.5 pound per animal daily. Some animals became weak, particularly in the hindquarters, and developed ataxia. A watery discharge from the eyes developed in some cases and nervousness in others. The test was repeated with two 2-month-old rabbits, and both died after 14 days.

*Dehydrated Leaf Meal*

For better control of feed consumption, a leaf meal was prepared. The leaves were hand-stripped 12 to 18 inches from the terminal portions of the vines, dried at 100° C., and ground in a hammer mill.

Twelve 2-month-old rabbits were divided into two groups of six animals. One group was used as a control and fed ground commercial rabbit pellets only. The other group was fed ground rabbit pellets and increasing amounts of leaf meal. During the first 4 days, 10 percent leaf meal was fed; 5 percent increases were made every 4 days until a level of 25 percent was reached. At this level the appetite became very poor. The level, therefore, was lowered to 20 percent, and for the last 20 days of the experiment to 15 percent. Feed consumption in the control group averaged 0.3 pound per animal daily, and in the experimental group 0.15 pound, up to the death of the first animal. After this, feed consumption averaged 0.08 pound per animal daily.

The control animals averaged a daily gain of 15 grams per animal during the 43 days of the experiment. The experimental animals lost weight. Two animals that lived through the experiment lost, on the average, 15 grams daily. Other animals in this group lost weight correspondingly.

During the experiment general health was good in the control group. In the experimental group, anorexia, emaciation, and, in later stages, general weaknesses developed. A serous discharge from the eyes was noticeable. In some advanced cases a corneal opacity developed. The first animal died after 18 days. The second was euthanatized after 27 days. The third died after 31 days. The fourth was euthanatized after 36 days. The fifth died after 46 days, and the last was euthanatized after 50 days. Euthanasia was performed to obtain fresh organ sections with no decomposition, since three of the animals became so weak that they were expected to die.

In a second experiment a ration with 20 percent leaf meal was again fed to six 2-month-old New Zealand White rabbits. The same symptoms developed, and all animals died within 31 days.

In a third experiment with 2-month-old rabbits the effect of creeping indigo upon the hemoglobin formation was studied. The plan was as follows:

1. Five rabbits were fed normal diet (rabbit pellets)
2. Five rabbits were fed 80 percent rabbit pellets  
20 percent creeping indigo leaf meal
3. Five rabbits were fed 75 percent rabbit pellets  
20 percent creeping indigo leaf meal  
5 percent ground limestone

The limestone was fed to determine whether the basic influence of calcium would affect the course of the disease. No such effect was noticed. The first animal (in group 3) died after 17 days, and the second (in group



2) died after 18 days. Remaining animals became so weak that they were euthanatized for collection of blood and urine (see table 5).

A fourth experiment studied the pathogenesis of creeping indigo poisoning as it developed in rabbits. Twenty healthy, 4-month-old, 4- to 5-pound New Zealand White rabbits were placed on a diet containing 20 percent leaf meal mixed into 80 percent ground commercial rabbit pellets. Two additional rabbits were fed ground commercial pellets only and kept as controls.

The rabbits were housed two to a cage, and two were killed each week. The original plan was to kill both rabbits from the same cage each week, but about 3 weeks after the feeding trials began, heavy mortality started and it became necessary to euthanatize from more than one cage.

In this trial, two rabbits were killed each week for 5 weeks, and 10 rabbits died as a direct result of creeping indigo poisoning.

Of the latter 10 rabbits, 7 died between the third and fourth week of feeding. One died after 18 days, one after 32 days, and one after 36 days.

The clinical syndrome was the same as mentioned before. The rabbits accepted the feed readily for 3 or 4 days and then developed anorexia. This was followed by progressive loss of weight and emaciation. They frequently developed ataxia shortly before death.

The 10 rabbits dying a natural death were severely emaciated. From an average weight of 4.3 pounds at the start of the feeding trials, they fell to an average of 2.9 pounds at the time of death.

Altogether, 42 rabbits were fed the 20 percent leaf meal ration. Twenty-one died and 21 were euthanatized for a close study of organs and tissues.

### PREGNANT DOES

*Effect of 10 percent indigo leaf Meal in ration.*—Thirteen does were divided into two groups:

1. Control, normal ration: Six animals. These were fed a ration of 90 percent rabbit pellets and 10-percent commercial calf feed.
2. Experimental group: Seven animals. These were fed a ration of 10 percent creeping indigo leaf meal, 80 percent rabbit pellets, and 10 percent commercial calf meal.

In the control group the does kindled with six to nine young per litter. The young lived and developed normally. The results of feeding the experimental ration are given in table 3.

TABLE 3. Result of feeding 10 percent creeping indigo leaf meal in normal ration to pregnant does.

NUMBER OF DOE	DAYS OF PREGNANCY WHILE ON INDIGO RATION	NUMBER OF YOUNG BORN	CONDITION OF YOUNG AT BIRTH
37	last 3	8	2 dead, 3 died second day, 3 lived
28	last 15	7	all dead
3	last 16	14	all dead
35	last 18	8	all dead
24	last 28	unknown	presumably all eaten
26	30	unknown	presumably all eaten
33	30	did not kindle	

Table 3 shows that 10 percent indigo leaf meal in the ration during the last 15 to 18 days of pregnancy was sufficient to produce stillbirths. It appears that even 3 days of feeding influenced the viability of the young. Some aftereffect could be noted, inasmuch as stillbirths occurred 37 to 45 days after feeding of leaf meal was discontinued. The first litter after the termination of the creeping indigo feeding was affected. In the second litter, there were no stillbirths.

*Effect of 5 percent indigo leaf meal in ration.*—The level of 5 percent leaf meal in a normal ration fed to does during the last 15 days of pregnancy also caused stillborn young (table 4).

TABLE 4. Result of feeding 5 percent creeping indigo leaf meal in normal ration to pregnant does.

NUMBER OF DOE	DAYS OF PREGNANCY WHILE ON INDIGO RATION	NUMBER OF YOUNG BORN	CONDITION OF YOUNG AT BIRTH
38	last 15	7	all dead
39	last 15	8	all dead
41	last 15	6	all dead
42	last 15	7	all dead
45	last 15	6	all dead

Five does included in the test delivered 34 young, all of which were dead. Two more does showed signs of delivery, but presumably the young were eaten by the mothers before any count could be made.

An interesting difference between results obtained for the 10 percent and the 5 percent levels was that the 10 percent level seemed to cause a detrimental aftereffect, and no such effect was found at the 5 percent level. Six does in the test gave birth to 35 normal young within 42 to 58 days after the feeding of 5 percent leaf meal was discontinued.

### GUINEA PIGS

In a brief study, nine young guinea pigs were divided into two groups with four and five animals per group. The weight of the pigs averaged 523 grams. The control group was fed green grass and a commercial calf ration ad libitum. This group developed normally; all animals were in good health and gained weight. The experimental group was fed fresh creeping indigo and a commercial calf ration ad libitum. The consumption of the commercial calf ration averaged 10 grams daily per animal. Early in the experiment the consumption of the fresh indigo was 0.1 pound daily per animal, but later less was consumed. After 3 weeks, the commercial calf ration was excluded from the diet and only fresh indigo was given. The symptoms were loss of appetite and emaciation. The animals appeared more shy and not as active as the guinea pigs in the normal group.

One animal became very weak and was euthanatized after 24 days. Another animal died after 35 days. The two remaining animals were euthanatized for histopathological studies after 35 days.

## POST-MORTEM RECORDS

*Cow*

One of a group of 16 bred dairy cows and heifers was fed unchopped, fresh creeping indigo as supplement to sparse pasture for 32 days. The cow ate about 20 pounds of the legume daily. A dead calf was produced 3 days after the indigo feeding ceased, and the cow died 5 days after calving. Autopsy revealed a marked protrusion of the vulva. There were numerous petechial hemorrhages of the subcutis, on the lung, and on the intestinal serosa. The myocardium contained gray striations. Cirrhosis of the liver was marked by a "hobnailed" surface and increased resistance to cutting. The uterus was enlarged and filled with fetid fluid. The uterine mucosa was decomposed. Tissue section of the liver revealed considerable post-mortem decomposition, but it was possible to see extensive interstitial connective tissue proliferation (chronic interstitial hepatitis). The myocardial sections were not studied because of the decomposition of the cells. Culture of the liver and spleen was negative for common pathogens.

*Sheep*

A Merino ram fed on fresh, chopped creeping indigo for 38 days was euthanatized for histopathological studies. Clinical examination revealed a general weakness and unsteady gait, atonic rumen, and partial bilateral corneal opacity. There was evidence of some sight. The animal was bled to death. Post-mortem examination revealed a fatty degeneration of the liver as the primary gross pathology. The rumen was filled with a watery fluid. When this was drained, an impacted mass of fibrous stems of creeping indigo was found, filling the organ to about one third of its capacity. The reticulum was full of the same material. The omasum was shrunken in size and empty. The abomasum was distended with fluid and coarsely ground fibrous ingesta, which tended to pack toward the pylorus. The small intestine was practically empty, containing little fluid or ingesta. The caecum appeared normal and was filled with normal-appearing ingesta. The large bowel was filled with a greenish fluid. When the brain case was opened, the meninges of the cerebrum were found to be congested. Weight of spleen was 47.1 grams. Examination of stained corneal smears from the areas of opacity did not show changes associated with avitaminosis A.

The second animal, a Corriedale ewe, was fed chopped creeping indigo for 26 days before death. Post-mortem examination revealed lesions similar to those mentioned above. There were also hemorrhages in the kidney pelvis. The gall bladder was abnormally large and filled with bile.

The third animal, a Suffolk ewe, was fed chopped creeping indigo and euthanatized after 38 days. Post-mortem examination revealed no lesions in addition to those mentioned above.

*Rabbits* (a representative case reported)

A 4-month-old New Zealand White buck on a diet containing 20 percent creeping indigo meal developed anorexia, progressive emaciation, and ataxia. It died after 3 weeks of experimental feeding.

At autopsy, the outstanding lesion was cirrhosis of the liver. Other findings were passive congestion of the lungs and kidneys.

Microscopically the liver sections contained an increased amount of portal connective tissue that was infiltrated with lymphocytes and monocytes.

The hepatic cells were large, granular, and sometimes vacuolated. Occasional islands of regenerating cells were present.

### PATHOLOGICAL AND HISTOPATHOLOGICAL STUDIES

Organ sections of 12 rabbits and 4 guinea pigs that had either died naturally or had been euthanatized after being fed fresh or dehydrated creeping indigo were studied by the Armed Forces Institute of Pathology, Washington, D. C.<sup>1</sup> The findings were summarized as follows:

A cirrhosis of undetermined type with regeneration of the hepatic cells and proliferation of bile ducts, biliary epithelium and periportal connective tissue was found consistently in all of the rabbits. The picture varied amongst different animals only in degree or intensity. Bile stasis was observed in two cases and moderate hemosiderosis in several. The picture in the guinea pigs was not very clear or consistent. A moderate fatty degeneration of the liver was observed in one case. The findings were not impressive in the remainder of the cases.

### OBSERVATIONS ON PATHOGENESIS

The fourth experiment with leaf meal, performed with 20 4-month-old rabbits fed 20 percent leaf meal, revealed the following observations:

The most frequent effects were emaciation, cirrhosis, hydrocephalus, passive congestion of the kidney medulla, and scattered areas of passive congestion and edema of the lungs. Eight of the 10 rabbits that died naturally showed cirrhosis. The liver varied from light yellow and finely granular to coarsely granular and mottled yellow and red in the indentations with a normal color in the raised areas. Two were slightly discolored by bile pigment. They did not show appreciably increased resistance to cutting.

Four animals developed hydrocephalus with dilatation of the lateral ventricles and pressure atrophy of the cerebrum.

Passive congestion of the kidney medulla was present in eight cases; the kidney was normal in size but slightly darker than normal in color. The medullary portion was a dark reddish blue, and blood oozed from the cut surface.

Four cases showed scattered areas of passive congestion and edema in the lungs. The lesions were more pronounced in the ventral portions of the anterior lobes. Edema was severe in only one case.

Microscopically, the normal lobular pattern of the liver was distorted by extensive connective tissue and bile duct proliferation around the portal triads. The proliferative tissue was often infiltrated with monocytes, lymphocytes, and plasma cells. Areas of hemorrhage and necrosis were occasionally seen in the same region. The hepatic cells were swollen, granular, and occasionally vacuolated. A few islands of regenerating hepatic cells were found and occasionally a single large cell was seen in the process of mitosis. Frequently the hepatic cells of the portal area contained dark brown granules of bile pigment.

Sections of the kidney showed only congestion of the straight veins of the medulla.

The alveoli of the affected areas of the lung were filled with erythrocytes and transudate. Numerous hemosiderin-laden monocytes (heart failure cells) were present. The alveolar capillaries were congested.

<sup>1</sup> Director: Colonel Elbert DeCoursey. Examination and report prepared by Major C. A. Gleiser.

Sections of the brain failed to reveal the cause of the hydrocephalus.

The first two rabbits fed 20 percent creeping indigo were killed 8 days after the feeding trials started. Lesions detectable at autopsy were confined to the liver and were the same in both cases.

The liver was normal in size and shape but contained light tan, pinhead-sized areas of albuminous degeneration scattered evenly throughout the surface and the cut surface. Microscopically, the portal hepatic cells were swollen, granular, and occasionally vacuolated. The portal veins were enlarged and filled with blood.

The two animals examined after the second week had finely granular livers. Microscopically, the lesions had progressed from degeneration to necrosis. The necrosis was not prominent, and in most areas the dead cells had been replaced by proliferating portal connective tissue and bile ducts.

Passive congestion of the kidneys and edema of the ventral portion of the anterior lobes of the lungs had developed. The microscopic lesions resembled those already described.

By the third week a frank emaciation had developed, and both rabbits examined had hydrocephalus.

The livers were coarsely granular and identical with those described in the deaths that occurred naturally.

Medullary congestion of the kidneys was present, but the lungs were grossly normal.

After the third week there were no significant new developments and the liver changes predominated. Between the third and fourth weeks, 7 of the remaining 13 animals had died. Therefore, the rabbits killed in the fourth, fifth, and sixth weeks had survived a dosage sufficient to kill the majority.

Of the 10 euthanatized rabbits, all showed liver pathology, 8 had medullary congestion of the kidneys, 3 had areas of passive congestion and edema in the lungs, and 2 had hydrocephalus. This study revealed, therefore, that the early liver changes were degenerative in nature and began during the first week of feeding. The hepatic cells in the portal region became swollen and granular, later vacuolated, and finally necrotic. The process started at the portal triads and extended toward the central vein.

By the end of the second week the lesions were becoming proliferative instead of degenerative in nature. The necrotic hepatic cells were being replaced by connective tissue and bile ducts.

By the third week the lesions were chiefly proliferative, with the normal lobular structure obscured by large formations of connective tissue and bile ducts.

Passive congestion of the kidneys and lungs developed during the second week. All cases of hydrocephalus developed from 18 to 25 days after feeding of creeping indigo started. After 3 weeks, no additional lesions were found. The control animals had no lesions.

## STUDIES ON BLOOD

At different periods of the year when creeping indigo was fed to the heifers in dry lot, blood samples were drawn and tests made for hemoglobin values, erythrocytes, leucocytes, blood sugar, serum calcium, and serum phosphorus. Simultaneously, blood samples were taken from heifers fed on

TABLE 5. Blood studies of heifers, sheep, and rabbits.

FEED	NO. OF TESTS	HEMOGLOBIN	ERYTHROCYTES	LEUCOCYTES	BLOOD SUGAR	SERUM CA	SERUM P
		<i>gm./100 cc.</i>	<i>millions</i>	<i>thousands</i>	<i>mg./100 ml.</i>	<i>mg./100 ml.</i>	<i>mg./100 ml.</i>
<i>Heifers</i>							
Creeping indigo, dry lot	8	12.93 ± 0.61	7.63 ± 0.22	7.46 ± 0.33	57.2 ± 3.76	10.0 ± 0.26	8.3 ± 0.29
Creeping indigo, pasture*	3	12.80 ± 0.35	7.32 ± 0.29				
Control	3	11.6 ± 0.40	6.42 ± 0.35	7.43 ± 0.38	60.1 ± 0.64	10.8 ± 0.37	8.1 ± 0.10
Normal range, cattle	..	8.0 — 14.5	5.0 — 10.3	5.0 — 12.0	40 — 60	9.0 — 12.0	2.3 — 9.6
<i>Sheep</i>							
Creeping indigo, dry lot	6	14.3 ± 0.60	10.70 ± 0.47	8.59 ± 1.38	66.5 ± 3.42	9.2 ± 0.62	5.7 ± 1.02
Control	4	10.5 ± 0.96	9.11 ± 0.54	9.40 ± 0.83	59.2 ± 1.13	9.6 ± 0.55	9.0 ± 0.43
Normal range, sheep	..	9.0 — 14.5	9.0 — 14.0	4.0 — 10.0	40 — 65	9.0 — 12.0	2.5 — 9.0
		Hemoglobin					
		At start of experiment	After 11 days				
		<i>gm./100 cc.</i>	<i>gm./100 cc.</i>				
<i>Rabbits</i>							
Creeping indigo leaf meal 20 per cent.	5	11.7 ± 0.47	11.6 ± 0.71				
Creeping indigo leaf meal 20 per cent and 5 percent CaCO <sub>3</sub>	5	11.3 ± 0.35	11.3 ± 0.48				
Normal dry ration	5	10.6 ± 0.63	10.9 ± 0.25				

\* Hemoglobin tests of heifers on pasture were carried out or supervised by Dr. J. M. Hendershot, Deputy Veterinarian of the Territorial Board of Commissioners of Agriculture and Forestry. Other analytical work reported in this table was performed by Miss Atsuko Ogai, Laboratory Technician, Board of Agriculture and Forestry.

normal diet. Hemoglobin and erythrocyte counts were performed on the heifers kept on creeping indigo pasture.

Analytical methods used were Wong's for hemoglobin (18); Folin and Wu's for blood sugar (6); the Clark-Collip modification of the Kramer-Tisdall for serum calcium (Ca) (3); and Fiske and Subbarow's for serum phosphorus (P) (5).

In the experiments with sheep, similar determinations on blood constituents were carried out for the animals fed creeping indigo and also for the controls. Tests were performed before the experiment began and after 24 days of feeding.

Hemoglobin determinations on rabbits were carried out for rations with and without creeping indigo. Twenty percent leaf meal was fed in a dry ration. Hemoglobin determinations were made after 11 days on experiment.

The results of the blood studies are shown in table 5.

### STUDIES ON URINE

In studies of urine, the analytical methods used were for ammonia-nitrogen, modified Van Slyke and Cullen method (8); urea-nitrogen, modified Van Slyke and Cullen Urease Method (8); total nitrogen, Kjeldahl Method (8); and indican, Parker's modification of Askenstedt's method (8). Average results are collected in table 6. The observations were made for sheep and rabbits only.

#### *Amount of Urine*

The amount of urine excreted was about twice the normal in sheep and rabbits fed creeping indigo.

#### *Ammonia-Nitrogen*

The urine of the sheep fed creeping indigo contained a higher amount of ammonia-nitrogen than did the normal urine. In general, the amount per 100 cc. of urine, as well as the total amount excreted in 24 hours, was definitely increased. When 20 percent creeping indigo leaf meal was fed, there was a tendency to higher 24-hour output of ammonia-nitrogen although the amount per 100 cc. was less. However, for rabbits fed green fresh creeping indigo only, there was much more ammonia-nitrogen per 100 cc. as well as per 24 hours.

#### *Urea-Nitrogen*

In sheep, output of urea in urine was no higher when creeping indigo was fed. The concentration of urea per 100 cc. was less; but due to the higher amount of urine, the total daily output of urea was not much different. In rabbits the total output of urea, as the concentration per 100 cc. of urine, and the volume per 24 hours were higher than normal.

#### *Total Nitrogen*

For sheep and rabbits, the output of total nitrogen in urine per 24 hours was larger than normal. While the concentration was less, the high daily output of urine raised the total amount.

#### *Indican*

For sheep the total daily output of indican was larger than normal. There was no indican, or not more than a trace of it, in the urine of normal rabbits, while some indican was always present when creeping indigo was fed.

TABLE 6. Studies of urine in sheep and rabbits.

FEED	OBSER- VATIONS	URINE cc./24 hours	NH <sub>3</sub> -N (GM.)		UREA-N (GM.)		TOTAL N (GM.)		INDICAN (MG.)	
			per 100 cc.	per 24 hours	per 100 cc.	per 24 hours	per 100 cc.	per 24 hours	per 100 cc.	per 24 hours
<i>Sheep</i>	5	529	1.82	9.6	4.4	23.3	19.6	103.7	0.95	5.0
	5	1023	2.82	28.8	2.4	24.6	14.9	152.4	0.85	8.7
<i>Rabbits</i>	3	82	1.2	0.98	5.4	4.4	12.5	10.3	0-trace	0-trace
	3	183	0.7	1.3	6.4	11.7	10.4	19.0	1.3	2.4



## DISCUSSION

*Cattle*

The strain of *Indigofera endecaphylla* tested in Hawaii resulted in serious poisoning of cattle, sheep, and rabbits.

Feeding this legume to pregnant cattle results in dead or stillborn calves. Feeding the legume to appetite for 25 days causes abortion or dead calves. The calves were born at normal time or as much as 100 days prematurely. The abortive agent of the legume was present in the fresh forage utilized as pasture and in the harvested forage, either chopped or unchopped, until it was 2 to 3 days old and partially dried.

Another effect of feeding the harvested legume was a decreased appetite and, for certain periods, loss in weight. Periods of loss of weight lasted 1 to 2 months. The weight was in some cases regained, but one heifer suffered an almost complete 12-month cessation in growth. Conception took place even after 11 months of feeding the legume, but the feeding of indigo delayed the period of heat in the heifers. However, this may be a secondary effect resulting from subnutrition.

During 10 years of intermittent grazing trials with low levels of creeping indigo mixed with grasses, no adverse effect on appetite, growth, or nervous disorder was noted on nonbred heifers. When the pasture contained over 50 percent creeping indigo, however, detrimental effects developed. The present feeding trials have shown conclusively that creeping indigo, when consumed in sufficient quantity, is highly potent in causing abortion in pregnant cows. It should be mentioned that the abortions occurred in animals free from Bang's disease.

In a few instances the central nervous system was affected (1). The heifers seemed drowsy, walked in circles, and carried their heads low. Where such effects were noted, recovery followed after 1 or 2 days. However, as mentioned earlier, one cow died after she had been fed chopped creeping indigo for 33 days. Death came 5 days after delivery of a dead calf and was probably due to an untreated metritis and resultant toxemia.

*Sheep*

The indigo had a more pronounced poisonous effect on sheep than on cows. Two of the four sheep died after 26 to 28 days while the other two were so weak after 38 days that euthanasia had to be performed. One pregnant ewe produced a dead lamb after only 1 day of feeding on the legume, and it appears possible that this abortive effect was caused by the creeping indigo. The symptoms of the sheep were largely the same as those of the heifers—loss of appetite, loss in weight, and drowsiness. In addition, a serous discharge of the eyes, a purulent discharge from the nostrils, and, in later stages, corneal opacities were observed.

*Rabbits*

Creeping indigo had even more serious effect on pregnant does, as 5 percent indigo leaf meal in the diet during the last 15 days of pregnancy produced stillbirths. Furthermore, fresh creeping indigo fed ad libitum killed the animals within 7 to 30 days and 20 percent indigo leaf meal in dry ration killed rabbits within 30 to 50 days. These observations confirm the work of Emmell and Ritchey (4). The symptoms in the rabbits resembled those of the larger animals; at first they enjoyed good appetite, but later developed

anorexia, loss in weight, and emaciation. Still later they exhibited nervousness, paralysis of hindquarters, and ataxia, and occasionally a serous discharge of the eyes or opacity of the cornea.

The following symptoms, therefore, apply to heifers, sheep, and rabbits fed on the harvested legume: good appetite for the first few days with later loss of appetite, loss in weight, and abortive effect on pregnant females. A nervous effect was also noted: heifers walked in circles, sheep pressed their heads against the fence, and rabbits developed ataxia or partial paralysis. In sheep and several rabbits a serous discharge from the eyes and opacity of the cornea appeared. In sheep there was also a purulent nasal discharge and severe diarrhea. Hydrocephalus developed in several rabbits.

### *Body Organs*

The most pronounced effect on the vital organs was on the liver, where congestion, fatty degeneration, or beginning or fully developed cirrhosis was observed. Lesion of the liver was found in cattle, sheep, and rabbits. Other organs were also affected, but the effect on the liver was most outstanding. Udall describes cirrhosis of the liver as an increase in the connective tissue combined with a degeneration of the liver cells (16). It is clinically characterized by jaundice, deranged consciousness, and motor irritation. A hyper- or atrophic condition may exist. In the hypertrophic form the liver is reddish yellow or bronze and friable. The symptoms for cirrhosis are given by Udall (16) as a gradual loss in condition, leading to anorexia, debility, and alternating diarrhea and constipation without apparent cause. There is no fever. Among the nervous symptoms are deranged consciousness, such as marked dullness and somnolence, sometimes delirium, or the development of motor irritation assuming the form of muscular twitching, aimless walking in circles, pressing the head against objects, etc. In advanced cases there may be paralysis. Due to the variety of causes, the symptoms may vary widely. Other descriptions of the gross appearance of cirrhosis are given by Bell (1) and Boyd (2).

Most of the symptoms mentioned above were noted in our experiments. However, cirrhosis is by no means a disease that is specifically the effect of creeping indigo. In 1892, cirrhosis of the liver was described as occurring among horses in the lower Missouri Valley (14, 15), and the cause was undetermined. In 1893, Johnson (9) described an enzootic of hepatic cirrhosis in pastured cattle in Nova Scotia. The farmers attributed it to eating ragwort (*Senecio jacobaeus*). In 1900, Gilruth described hepatic cirrhosis in horses and cattle in New Zealand. Here also it was attributed to ragwort, and Gilruth caused cirrhosis to develop in two 6-month-old calves by feeding them *S. jacobaeus* (7). In 1929, Van Es and coworkers (17) reported on "walking disease" with liver cirrhosis among horses and cattle during June and July in northwestern Nebraska. The cause proved to be the plant *Senecio riddeli*, which occurred in the pasture. In Florida, Sanders (13) reported cirrhosis of the liver in steers as caused by eating *Crotalaria spectabilis*. In the Pacific Northwest, McCulloch (10) reported hepatic cirrhosis (walking disease) of horses, swine, and cattle as caused by the seeds of yellow tarweed (*Amsinckia intermedia*).

In our investigation, lesions noted on other organs were alternate striations of gray muscle and normal tissue in the cow's heart and some areas of degeneration and striations in the rabbits' hearts. Passive medullary con-

gestion was noted in the rabbits' kidneys. Petechial hemorrhages occurred under the surface of the lungs of the cow and the sheep, and edema with passive congestion in the rabbits. However, the organ most severely affected was always the liver.

### *Hemoglobin*

In heifers the daily consumption of about 20 pounds of creeping indigo did not affect the hemoglobin content of the blood. The results obtained were all within the normal range (table 5). The same condition was true for the sheep. In this instance the amount of the legume consumed varied from less than 1 pound to 4 pounds daily. Hemoglobin values averaged 14.3 grams per 100 cc. blood. In one sheep, blood samples were taken 5 days before death. The hemoglobin content of that sheep was 15.6 grams per 100 cc. The hemoglobin content of an animal weak and close to death was 13.2 grams per 100 cc.

Similarly, no anemic condition was observed in rabbits after feeding creeping indigo. The hemoglobin values were normal after 11 days of feeding creeping indigo, 5 and 6 days before the animals started to succumb (table 5).

### *Blood Cells*

The values determined for erythrocytes and leucocytes in heifers and sheep were also normal. In our experiment the creeping indigo had no anemic effect on the animals.

In reports from Florida, Emmell and Ritchey (4) state that they found reduction in hemoglobin readings and, in some cases, lowered erythrocyte count and leucopenia when creeping indigo was fed to rabbits.

This difference between the rabbits in the tests in Florida and those in ours was due to a lower intake of the legume in our experiments. In the rabbits tested for hemoglobin, only 20 percent leaf meal was fed in dry ration. Emmell and Ritchey do not mention how much of the legume they fed to their animals but state that in addition to being allowed to graze the plant the rabbits were given small amounts of commercial feed daily.

It should be noted, however, that while 20 percent creeping indigo leaf meal in the ration was sufficient to kill the rabbits in our experiment, it had no anemic influence.

### *Glucose*

Creeping indigo feeding had no effect on the blood sugar level of heifers (table 5). There was an indication of increase in the blood sugar values in sheep, but the difference between controls and experimental animals is not statistically significant; the P value equals 0.2.

### *Calcium and Phosphorus*

Serum calcium was not influenced by feeding creeping indigo to heifers and sheep. The values of 10.0 mg. per 100 ml. for the heifers and 9.2 mg. for the sheep fall well within the normal range.

For the serum phosphorus there was a trend toward decrease of values in both heifers and sheep. In the heifers the average phosphorus values were 9.0 mg. per 100 ml. in January and 7.1 in June. This decrease showed significance at the 5 percent point ( $P=0.05$ ). Although this drop is slight and does not appear in the averages given in table 5, it is worth noting. For the

sheep a similar situation occurred. The difference between the values of 5.7 for sheep fed creeping indigo and 9.0 for the controls is significant at the 5 percent point. Therefore, a small decrease in serum phosphorus occurred in connection with the feeding of creeping indigo. Whether this was a primary effect caused by an agent in the legume or a secondary effect due to the decreased feed intake is not known.

### Urine

The observations made on urine from sheep and rabbits are sufficient to show that the protein metabolism was affected in creeping indigo feeding. Results noted are increases in ammonia-nitrogen and in total nitrogen and in the excretion of indican in urine.

The cause of the increased amount of urinary ammonia-nitrogen needs comment. Peters and Van Slyke mention (12) that such increase could be due to increased protein consumption or might also be considered as a defense against acidosis. Inasmuch as there was no increased protein consumption in this experiment,\* it appears possible that the increased urinary ammonia was attributed to acidosis caused by the creeping indigo. The pH values on urine from rabbits on normal feed were not less than 7.7 and usually ranged between 8 and 9, but for rabbits fed fresh creeping indigo pH values of 6.0 and 6.5 were noted.

The significance of the increased amount of indican in urine with creeping indigo feeding is not clear. Peters and Van Slyke (12) mention the possibility that indole is formed in the body as an oxidation product of tryptophane.

### SUMMARY

A study of the effect of a strain of *Indigofera endecaphylla* Jacq. (creeping indigo) grown in Hawaii and tested as a feed for cows, heifers, sheep, and rabbits is presented. The following results were obtained:

1. *Indigofera endecaphylla* used as pasturage in mixture with grasses, or harvested, chopped, and fed ad libitum in a fresh or semi-dry state, caused abortion or stillbirth in pregnant cows and heifers. The calves were born at normal time or prematurely by as much as 100 days. The abortive effect was found also in pregnant does fed as little as 5 percent dry leaf meal in dry ration.
2. Other symptoms caused by feeding the legume were anorexia, loss of weight, and emaciation. In advanced cases a motor nerve disturbance was manifest: heifers walked in circles; sheep pressed their heads against the fence; rabbits suffered ataxia. In sheep and rabbits a serous discharge from the eyes was observed, and occasionally opacity of the cornea developed. In sheep a purulent nasal discharge and severe diarrhea were noticed.
3. Feeding the fresh chopped legume to sheep for 28 days killed some of the sheep, while others became so weak that euthanasia was performed. Feeding the fresh legume killed rabbits within 7 to 30 days. Twenty percent dry leaf meal added to a normal ration killed the rabbits within 30 to 50 days.
4. The most outstanding effect on vital organs was on the liver. Disturb-

\* The rabbit pellets contained 17.5 percent protein and the creeping indigo leaves 17.1 percent protein on dry matter basis.

ances in the heart, kidneys, and lungs were also noted. In the liver, congestion, fatty degeneration, and cirrhosis occurred. Liver lesions were noticed in an autopsied cow, in a sheep, and in 48 rabbits. Hydrocephalus was found in several rabbits.

5. A study of the pathogenesis in rabbits was made. The early liver changes were degenerative and began during the first week of feeding. The hepatic cells in the portal region became swollen and granular, later vacuolated, and finally necrotic. The process started at the portal triads and extended toward the central vein. By the end of the second week, the lesions were becoming proliferative instead of degenerative in nature. The necrotic hepatic cells were being replaced by connective tissue and bile ducts. By the third week the lesions were chiefly proliferative, with the normal lobular structure obscured by large formations of connective tissue and bile ducts. Passive congestion of the kidneys and lungs developed during the second week of feeding. All cases of hydrocephalus developed between 18 and 25 days after the feeding of creeping indigo was started. After 3 weeks no additional lesions were found.
6. No effect of the legume on hemoglobin could be established in heifers, sheep, and rabbits. Neither was there any effect noted on erythrocytes, leucocytes, blood sugar, and blood calcium in heifers and sheep. A slight decrease in serum phosphorus was noticed for heifers and sheep.
7. A study of the urine in sheep and rabbits revealed increased amounts of urinary ammonia, total nitrogen, and indican, and a decrease in pH values.

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UNIVERSITY OF HAWAII  
COLLEGE OF AGRICULTURE  
AGRICULTURAL EXPERIMENT STATION

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GREGG M. SINCLAIR  
President of the University

H. A. WADSWORTH  
Dean of the College and  
Director of the Experiment Station

L. A. HENKE  
Associate Director of the Experiment Station